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V. M. Karasik (1945, 1946 and 1948) has drawn attention to the fact that the "affinity" strength of various substances to cholinesterase gradually diminishes, with a change from a substance with a "cholin-sensitizing" action (eserine, proserine) to a "cholinolytic" action (atropine), and subsequently to a "cholinomimetic" one (carbocholine). Considering cholinesterase as a model of choline-receptive substances in tissues, he proposed, that one or another variant in the effect (sensitizing, lytic, or mimetic) might depend on the varying strength of affinity of the pharmacological agent to the receptors of the cholinurgic structure.

Dyablova (1947-1948), in the laboratory of V. M. Karasik, observed all three variations with a gradual increase in the concentration of one and the same substance -- proserine -- in experiments with skeletal muscle.

In subsequent studies of the reactions of cholinurgic structures interest arose in the investigation of the action of various concentrations of proserine on muscles contracted under the influence of a stable cholinemimetic agent, not hydrolyzed by cholinesterase, as for instance carbocholine.

We already possessed a certain experimental basis for such experiments. With regard to eserine, it has been known for a long time that this substance may cause increased contraction in the back muscle of the leech under the influence of nicotine, muscarine, neurine, betaine, barium salts and certain other substances. Rybolovlev, in the laboratory of N. V. Lazarev, noted (1948) that proserine sensitizes the straight muscle of the frog abdomen to carbochloine.

THE INFLUENCE OF PROSERINE ON THE EFFECTS OF CARBOCHOLINE

Experiments on the Straight Muscle of the Frog Abdomen

The actions of proserine by itself are exactly those described by Dyablova. Fast, spontaneous tremors appear in muscles with the action of proserine (P) in concentrations of $1x10^{-6}$ to $2x10^{-5}$. Concentrations of $5x10^{-5}$, and higher stop this spontaneous activity. Starting at this same concentration, proserine starts to produce a slowly developing contracture of the muscle, which is especially clear with a subsequent increase in the proserine concentration to $2x10^{-4}$-$1x10^{-3}$.

The reactions of muscle to carbocholine (K) of $1x10^{-7}$- $5x10^{-7}$ are not altered at all with the action of proserine in concentrations lower than $1x10^{-6}$. Starting with this concentration, to a concentration of $1x10^{-4}$ inclusively, there is usually observed, in agreement with the data of Rybolovlev, some strenghtening of the reaction to carbocholine (sensitizing effect). A more distinct facilitation was observed with sustaining concentrations of carbocholine and proserine concentrations of $1x10^{-5}$, with extended recording of each shortening (Fig. 1, A). However, with concentrations of $1x10^{-4}$ to $1x10^{-3}$ proserine

lowered the height of the carbocholine contraction (lytic effect). The depression of carbocholine contraction by proserine is more evident with strong contractures caused by the carbocholine alone (Fig. 1,B). These same concentrations of proserine alone ($1x10^{-4}$-$1x10^{-3}$) cause a slow contracture of the muscle (mimetic effect, Fig. 1, B).

Fig. 1. The influence of proserine (P) on the sensitivity of straight abdominal muscle of the frog to carbocholine. A -- the sensitization of muscle to carbocholine by proserine in small concentrations, recorded on a rotating drum with each contraction during 60 minutes with periodic stopping; B -- the influence of high concentrations of proserine on the contraction of muscle with a lowering of its sensitivity to carbocholine, recorded on a stationary drum with each contraction over 5 min; carbocholine in a concentration of $5x10^{-7}$, proserine in a concentration of $1x10^{-4}$.

Experiments on Dennervated Leech Muscle

Proserine alone, used in concentrations of $1x10^{-8}$ to $1x10^{-4}$ produces no effect. Sensitization to carbocholine is observed irregularly with the action of proserine in concentrations of $1x10^{-7}$, and is expressed weakly. The lytic effect is observed fairly regularly with the action of proserine in a concentration of $1x10^{-6}$ and was ~~more~~ most pronounced with concentrations of proserine from $1x10^{-5}$ to $1x10^{-4}$. In this case the proserine often completely depressed the carbocholine contraction (Fig. 2).

Fig. 2. The depression of the reaction of the back muscle of the leech to carbocholine by proserine. Recorded on a rotating drum for each

contraction over the course of 5 minutes. Carbocholine in a concentration of 3×10^{-8}, proserine in a concentration of 1×10^{-4}.

Consequently, the experiments with leech muscle ~~of~~ 6 also demonstrate that proserine may change the reaction of muscle to a cholinemimetic agent, independent 17 of possible inhibition by choline-esterase, since carbocholine is not hydrolyzed by this enzyme.

It is possible that ~~the action~~ of proserine may have an analogous action with respect ~~relationship~~ to acetylcholine, but in direct experiments this "non antienzymatic" action cannot be detected, since it is masked by the much stronger "anti-cholinesterase" effect.

It is generally known, that the depression of cholinesterase activity with proserine may increase the sensitivity of leech muscle to acetylcholine by more than 10,000 times. At the same time, small changes in sensitivity to acetylcholine (2-3 times), which are observed as a result of the "non anticholinesterase" action of proserine on the cholinergic structure of muscle, may fully mask its strong sensitization to acetylcholine, based on the inhibition of cholinesterase by proserine.

In order to show this "non antienzymatic" action of proserine on the effect of acetylcholine, experiments were made with preparations of leech whose cholinesterase had been previously depressed with eserine.

THE ACTION OF PROSERINE ON THE SENSITIVITY OF ESERINIZED LEECH MUSCLE TO ACETYLCHOLINE

The optimal concentration of eserine for the sensitization of leech muscle to acetylcholine is 2×10^{-6}. With eserine present in

this concentration, the effects of various concentrations of proserine on the sensitivity of leech muscle preparations to acetylchoine were studied.

In concentrations of $1x10^{-6}$ to $1x10^{-5}$ proserine causes practically no change in the sensitivity of eserinized muscle to acetylcholine; though in some cases a slight potentiating effect was observed. In concentrations of $1x10^{-4}$ proserine sharply depressed acetylcholine contraction, not infrequently to its complete disappearance (Fig. 3) -- an unequivocal cholinelytic effect, evidently independent of the anti-cholinesterase action of proserine.

Fig. 3. Depression of the reaction of eserinized muscle (E) to acetylcholine (AKh) by proserine. Acetylcholine in a concentration of $5x10^{-9}$, eserine in a concentration of $2x10^{-6}$, proserine in a concentration of $1x10^{-4}$. Recorded on a rotating drum. The numbers over each line indicate the duration of the record in minutes.

In the reverse variation of the experiment, eserine in all used concentrations ($2x10^{-6}$-$1x10^{-4}$) either slightly facilitated the action of acetylcholine on proserinized leech muscle, or did not change it at all. No lytic action could be shown in this variation of the experiment.

Smirnov (1947) noted that frog muscle treated with diisopropyl-fluorophosphate showed a decrease in reaction to acetylcholine when treated with eserine in high concentrations.

SURVEY OF RESULTS AND CONCLUSIONS

We set up experiments with such conditions that proserine depression of the enzymatic breakdown of acetylcholine should not influence the results. None the less, proserine changed the reactions of leech and frog muscles to carbocholine and the reactions of eserinized leech muscle to acetylcholine. The results obtained verify that the action of proserine on cholinurgic processes cannot be fully attributed to the depression of enzymatic breakdown of acetylcholine (the detailed mechanism of the action of eserine and proserine were discussed by one of us elsewhere -- Mikhel'son, 1948). Proserine shows an action on enzymes in very small concentrations, but in addition, it acts on the ability of cholinurgic structrues to be influenced by other substances, and possibly, on their contractile properties.

Depending on various conditions (concentration of acting substances, the object, and others), the reaction of a cholinurgic structure to one substance may variously change the ability of this structure to react to other substances.

This point of view makes it easier to understand the most diverse effects of combining the actions of two substances on a cholinurgic structure, in particular -- the sensitization of leech muscle to carbocholine, which we have noted during the course of this treatise, or the sensitizing of the straight muscle of the frog abdomen to acetylcholine and carbocholine by the action of urethane, noted in the Karasik laboratory earlier (Mikhel'son, 1943).

SUMMARY

1. Proserine, in various concentrations, shows different actions on the carbocholine contracture of frog and leech muscle and on the

acetylcholine contracture of eserinized leech muscle. In low concentrations proserine facilitiates this contracture (cholin-sensitizing effect), while in high concentrations it depresses it (cholinolytic effect); it may also cause contraction itself (cholinomimetic effect).

2. The described phenomena cannot be explained by the anti-cholinesterase action of proserine.

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